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Adhesins and Ligands Involved in the Interaction of *Candida* spp. with Epithelial and Endothelial Surfaces

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INTRODUCTION

Clinical Relevance

Candida albicans and related species are the leading cause of disseminated fungal infection in the immunocompromised host, the diabetic, the neonate, and the postoperative patient. In patients with chronic mucocutaneous candidiasis, *C. albicans* causes an erosive dermatitis involving skin and mucous membranes and severe inflammation of the vaginal tract. Even in immunologically competent hosts, colonization can occur at epithelial surfaces, including the mouth, the perineum, and the vagina; when epithelial barriers are breached by implanted catheters or as a result of burns or surgical procedures, colonizing candidal species can then invade the host.

Adhesion of candidal species to the epithelium of the gastrointestinal or genitourinary tract therefore stands as a critical first step in the pathogenesis of candidal infection. Having colonized the host's mucosal surfaces, *Candida* species may then invade beneath the mucosal barrier into the vascular space, where continued replication causes hematogenously disseminated disease. Invasion through the endothelial barrier may then give rise to distant metastases at sites including brain, retina, endocardium, liver, spleen, kidney, and bone.

Several recent and excellent reviews have summarized a number of aspects thought to bear on candidal adherence, including the biochemistry of the cell wall (14), the adhesive characteristics of candidal mannoproteins (25), the role of hydrophobicity (24), and the effects of dimorphism (81). This review will focus entirely on mechanisms regulating adhesion of *Candida* species to epithelial and endothelial cells. Adhesion to splenic phagocytes, to fibrin-platelet matrices, or to plastic polymers will not be covered, since these topics have received thorough treatment elsewhere (24, 54, 89).

Numerous clinical studies have suggested a pathogenetic link between adhesion and colonization in vivo and subsequent invasion. For example, *C. albicans* constitutes 68% of isolates from sites other than blood in cancer patients, while *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* account for 12.3, 10.3, 3.0, and 1.5% of isolates, respectively (55). In representative studies of fungemia in immunocompromised hosts, diabetics, neonates, and surgical patients, *C. albicans* again accounts for 60 to 80% of isolates, while other *Candida* species are identified less than 20% of the time (13, 19, 46, 69, 73, 87). Other yeasts, such as *Saccharomyces cerevisiae*, are even less frequently implicated in colonization and invasive disease (30). On balance, the correlation between colonization and invasion indicates that the invasive potential of *Candida* species appears to reflect each species' relative ability to attach to the human host.

Generalizations and Caveats

Three types of adhesive interactions, each of which will be detailed below, have been proposed (14).

(i) Protein-protein interactions are those in which a protein on the candidal surface recognizes a protein or peptide

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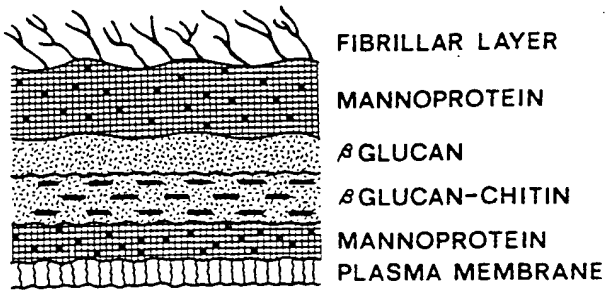


FIG. 1. Schematic diagram of the six layers of the candidal cell wall. Reprinted from reference 14 with permission of the publisher.

ligand on the epithelial or endothelial cell. This type of interaction is best exemplified by integrin-mediated adhesion.

(ii) Lectin-like interactions are interactions in which a protein on the candidal surface recognizes a carbohydrate on the epithelial or endothelial cell. This type of interaction is represented by candidal mannoproteins, which recognize a variety of carbohydrates on blood group antigens.

(iii) Incompletely defined interactions, in which a known surface component of *C. albicans* attaches to epithelial or endothelial surfaces by an as yet unidentified ligand, are the third type of interaction. Examples of these interactions include the putative role of the factor 6 epitope from *C. albicans* serotype A and the possible function of *C. albicans* aspartyl proteinase in degrading epithelial surfaces and promoting penetration of the fungus.

Despite the biochemical distinctions implicit in these adhesive interactions, there is general agreement on several fundamental aspects of candidal adhesion. First, with the exception of factor 6, virtually all adhesins identified to date are surface proteins, probably mannoproteins, as demonstrated by their susceptibility to various proteolytic enzymes such as trypsin, pronase, and zymolyase. The candidal cell wall consists of approximately six layers (Fig. 1), and mannoproteins are thought to be distributed throughout the layers (14). Circumstantial evidence suggests that a number of putative adhesins reside in the outermost fibrillar layer. Second, it is clear that viable yeast cells adhere more avidly than heat- or formalin-killed organisms (56, 101), suggesting the involvement of surface proteins susceptible to denaturation by heat or chemicals. Third, pseudohyphae and true hyphae are typically more adhesive than blastospores of the same isolate (56, 94). This observation suggests that candidal adhesins in hyphal phase may be more numerous, more avidly binding, or structurally altered so as to exhibit greater numbers of binding sites. Difficulties in the enumeration of germinated yeasts and the significantly greater hydrophobicity of hyphal forms, however, make direct comparison difficult.

Other factors that may introduce confounding variables in studies of adhesion include (i) the species of *Candida* under study, (ii) cell surface hydrophobicity of the fungus and the surface to which it will adhere, (iii) components of the fungal growth medium, and (iv) the type of assay employed. Not surprisingly, when one considers the incidence of various *Candida* species as etiologic agents of disease, *C. albicans* is significantly more adherent to buccal, vaginal, or HeLa cells (5, 21, 57); to skin corneocytes (85); to fibrin-platelet matrices (67); or to fibronectin (16, 63). Tissue specificity may also play a role in that adhesion mechanisms and exposed ligands appear to vary among different types of epithelial cells and between epithelial and endothelial cells.

The degree to which hydrophobicity influences interactions may vary with the system. Hydrophobicity is clearly an important determinant in the adhesion of *Candida* spp. to plastic, for example, and germinated forms of *Candida* spp., forms that generally are more adherent than blastospores, are universally hydrophobic (44). However, a statistically significant correlation between expression of cell surface hydrophobicity and adhesion to HeLa cells has not been found. Hydrophobicity was greatest for stationary-phase blastospores grown at 23°C, as opposed to 37°C, but only about 85% of the *C. albicans* strains tested fell into that category (41, 43). It would appear, therefore, that hydrophobicity is more important in adhesion to plastic than to epithelial cells.

The addition of various carbon sources to growth media has led to divergent results. For example, preincubation of yeast cells with fucose inhibited their adhesion to vaginal and buccal epithelial cells, whereas glucose, mannose, and galactose had no effect (101). Others have shown that 250 mM sucrose increased candidal adhesion to HeLa cells, but lesser concentrations of sucrose were ineffective (94). More recent studies of candidal adhesion to exfoliated buccal epithelial cells reported increased adhesion in the presence of 500 mM galactose versus 50 mM D-glucose (21). However, the presence of high concentrations of galactose is known to augment synthesis of the fibrillar layer, increase mannoprotein content, and enhance hydrophobicity (25), phenomena that may contribute to increased adhesion. In contrast, pretreatment of *C. albicans* with 200 mM concentrations of amino sugars such as mannosamine, glucosamine, and galactosamine inhibited adhesion to buccal cells. These results were not especially dramatic, however, since only two to three yeast cells adhered in the absence of sugars (18). Another example of the influence of carbon source on candidal infection compared colonization and invasion in neutropenic mice given drinking water supplemented with either 277 mM D-glucose or equimolar xylitol (107). Although plasma concentrations of glucose were not measured, the glucose-supplemented animals had a carbohydrate intake eight times that of the xylitol-supplemented animals and exhibited a significantly higher rate of gastric colonization and invasion than did the xylitol-supplemented animals. No mechanism for these effects was proposed. It is important to point out, of course, that physiologic concentrations of glucose range from 5 to 10 mM in the normal host and from 15 to 30 mM in the severe diabetic, although urinary concentrations may approach 50 mM (47); thus, some glucose concentrations employed in vitro may have little relevance in vivo.

The use of nonspecific inhibitors in the medium, such as detergents, reducing agents, proteinases, or EDTA, while supporting a role for protein-protein interactions in adhesion, has typically not yielded more definitive information (66).

The type of assay employed is critical to the outcome of adhesion studies and has frequently contributed to discrepant results found in the literature. Assays in which swabbed buccal or vaginal epithelial cells are used rely upon subjective enumeration of adherent yeasts, typically by Giemsa staining and microscopy. In assays in which exfoliated cells are used, differences in donor blood type, which determine expression of epithelial glycosides, may also impair reproducibility. Radiometric assays with labeled yeasts and a defined, cultured cell line offer considerably greater objec-

TABLE 1. Epithelial and endothelial adhesins

Adhesin	M_r (10^3)	Epithelial ligand	Inhibitor(s)	Gene cloned	Reference(s)
Epithelial					
Protein-protein					
Integrin analog (iC3b receptor)	130–165	iC3b	MAb, iC3b, RGD peptides	No	5, 27, 29, 37, 39, 45, 49, 82
Fibronectin	60–68	Fibronectin	Fibronectin, proteases	No	100
Lectin-like					
Fucose-binding protein	ND[a]	Fucose	ND	No	22, 23, 25, 70, 72, 105
GlcNAc-binding protein	ND	*N*-Acetylglucosamine	ND	No	22, 23, 25
Partially determined					
Aspartyl proteinase	45	ND	Pepstatin	Yes	36, 50, 90–92, 103, 110
Factor 6	ND	ND	MAbs, polyclonal antibodies	No	75, 76
Endothelial (protein-protein)					
Integrin analog	130–165	iC3b	MAb, iC3b, RGD peptides	No	28, 39
Fibronectin	60–68	Fibronectin	Fibronectin, RGD peptides	No	60–64, 97, 98, 100
Laminin	60–68	ND	Laminin	No	10
Fibrinogen-binding protein	60–68	ND	Protease, mercaptoethanol	No	8, 9, 17, 106

[a] ND, not determined.

tivity, provided that nonspecific adhesion is addressed in parallel studies with unlabeled organisms and that the cell line of choice has at least nominal relevance (5, 39, 42, 93). Pour plates or agar overlays are perhaps the most precise for the enumeration of adherent organisms, but they are hampered by the fact that the inoculum is typically low (10^2 to 10^3 CFU) in order to visualize individual colonies (60, 61). An enzyme-linked immunosorbent assay (ELISA) that uses a rabbit antiserum to *C. albicans* has also been proposed to be an objective measure of candidal adherence to endothelial monolayers in fibronectin-coated microtiter wells (31), and detachable filters for collagen matrices that support endothelial monolayers have been employed to obviate the difficulties of removing adherent yeasts and cellular monolayers (71). Predictably, adhesive ligands already identified on mammalian cells are either proteins or carbohydrates that are expressed on the cell surface. Failure to identify the adhesive ligand on the mammalian cell, however, makes the case for any candidate candidal adhesin considerably less compelling. Thus, the identification of a putative adhesin on the candidal cell should be coupled with vigorous attempts to elucidate its mammalian ligand and to confirm the specificity of the interaction.

EPITHELIAL ADHESION

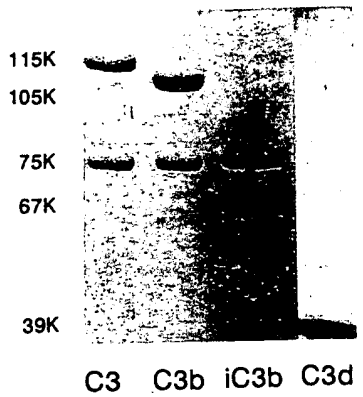
As introduced above, adhesin-ligand interactions have been divided into three categories for the purposes of this discussion: (i) protein-protein interactions, two examples of which are the integrin analog (iC3b receptor or CR3-like protein) and the fibronectin receptor; (ii) lectin-like interactions, including mannoproteins recognizing fucose or *N*-acetylglucosamine; and (iii) incompletely defined interactions, such as those involving secretory aspartyl proteinase or factor 6.

A number of parameters have been tabulated for the various epithelial and endothelial adhesins proposed to date (Table 1). Criteria used to evaluate candidate adhesins should include the use of specific inhibitors to block adhesion and the identification of the epithelial ligand. Both considerations are critical when the significance of the data is assessed.

Protein-Protein Interactions

Integrin analogs. Candidal proteins exhibiting antigenic and functional similarities to human complement receptors 3 and 4 (CR3 and CR4) are now known as integrin analogs because of the placement of CR3 and CR4 within the integrin supergene family (20, 95). Because the nomenclature for C3 fragments (C3b, iC3b, and C3d) and their corresponding receptors (CR1 to CR4) may be confusing, a figure has been included (Fig. 2). The mammalian integrins, which make up a gene family of 14 α-chains and 8 β-chains, are expressed as heterodimeric transmembrane proteins on a wide variety of mammalian cells, including epithelial and endothelial cells and leukocytes (51, 58). Integrins are currently grouped in subsets on the basis of their β-chains. As shown in the schematic diagram (Fig. 3), both α- and β-chains have cytosolic tails that mediate intracellular signaling via interactions with actin and talin, both chains have a highly conserved transmembrane domain that is specific for the α or β lineage, and each chain extends outward in a large extracellular domain. It is presently thought that the extracellular domains of both the α- and β-chains cooperate in the recognition and binding of integrin ligands such as fibrinogen, fibronectin, and laminin in the extracellular matrix. Many, but not all, of these ligands are distinguished by the tripeptide sequence arginine-glycine-aspartic acid, or RGD.

Several motifs are common to many integrins. First, the three cation-binding sites in the α-chain, which carry the consensus sequence DxDxDGxxDxxxGAP, have been found in several integrins (20). These three sites are thought to be important in ligand recognition, which is calcium dependent in the mammalian integrins (20). However, as noted below, recognition of integrin ligands by *C. albicans* is cation independent; therefore, one might not necessarily expect to find these sites in integrin analogs in yeasts. Second, the heavily cysteinylated carboxy terminus is a motif common to all β-chains so far described, save for an unusual β8 chain from rabbit and human placenta (79). Third, the hydrophobic transmembrane domains in several α-chains contain conserved glycine and leucine residues, as well as the sequence GFFKR. In the β-chains, leucyl residues typically make up only 20% of the transmembrane



COMPLEMENT RECEPTORS

Receptor	C3 Ligand	Mammalian Cells
CR1	C3b	PMN's, macrophages, monocytes, lymphocytes, RBC's, renal podocytes
CR2	C3d	B lymphocytes
CR3	iC3b	PMN's, macrophages, monocytes, NK cells
CR4	iC3b	PMN's, macrophages, monocytes, NK cells

FIG. 2. Roster of receptors for fragments of the third component of complement, C3. The electrophoretic patterns of the purified ligands, C3b, iC3b, and C3d, are shown under reducing conditions. Integrin analogs in *C. albicans* share antigenic, structural, and functional homologies with CR3 and CR4. PMN's, polymorphonuclear leukocytes; RBC's, erythrocytes; NK, natural killer. Reprinted from reference 48 with permission of the publisher.

domain, and the carboxy sequence FExER is shared by four of the eight leucyl residues (79).

Two members of the β2 integrins share antigenic, structural, and functional homologies with surface determinants in *C. albicans* and related species: namely, α_M, which is also known as Mac-1, CD11b, or CR3; and α_X, which is known as p150,95, CD11c, or CR4.

α_M and α_X are polypeptides of 165 and 150 kDa, respectively; in mammals, they each combine with a common β-chain of 95 kDa. In the human genome, α_M and α_X reside on the short arm of chromosome 16, while the 95-kDa β2 chain resides on the long arm of chromosome 21 (58). A number of integrin monoclonal antibodies (MAbs) with specificities for α_M or α_X have been shown to be reactive with *C. albicans*. Table 2 lists their functional parameters,

β_2 INTEGRINS

		a	β
a_L	LFA-1/CD11a	190	95
a_M	Mac-1/CD11b/CR3	165	95
a_X	p150,95/CD11c/CR4	150	95

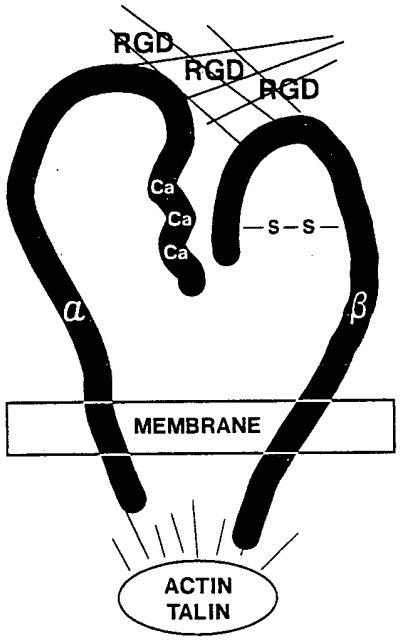


FIG. 3. Diagrammatic representation of α- and β-chains of the mammalian integrins. The amino termini are extracellular, the transmembrane domains are represented by the shaded areas, and the cytosolic tails are located at the carboxy termini, where they interact with actin and talin. RGD represents the tripeptide sequence arginine-glycine-aspartic acid, which occurs in many integrin ligands found in the extracellular matrix. Each Ca represents a cation-binding site. —S—S— represents the intrachain disulfide bond in the β-chain.

including their reactivity with isolates of *C. albicans* on flow cytometry and their relative abilities to block rosetting of iC3b-coated erythrocytes and adhesion of *C. albicans* to epithelial cells (5, 27, 29, 37, 45). Of the integrin MAbs tested, only Leu-15, directed against α_M, failed to bind to *C. albicans* (29). Thus, a number of MAbs recognizing the α-chains of β2 integrins (α_M and α_X or CR3 and CR4) bind to *C. albicans*; in addition, two of these are known to inhibit integrin-mediated adhesion to epithelial and endothelial surfaces (5, 39). Interestingly, the efficacy with which MAbs to other complement receptors (e.g., CR2) bind to *C. albicans* is not so predictable. Of the seven antibodies examined in the literature, three bind to *C. albicans*, (Table 2) whereas 2G7, 6F7, 1C8, and 3F11 do not.

The presence of integrin analogs in candidal species gives rise to two important biological correlates. First, because integrins on mammalian cells recognize RGD-containing ligands in extracellular matrices, integrin analogs on yeasts may well mediate adhesion by the same biochemical mechanism. Second, evidence of a significant relationship between integrin analogs in yeasts and their counterparts in mammalian cells could provide critical insights into the evolution and function of these complex eukaryotic proteins.

The first evidence for integrin analogs in *Candida* spp. was

TABLE 2. Binding of MAbs to *C. albicans*[a]

Specificity	MAb	Isotype	Reactivity with *C. albicans*	Protein(s) recognized (kDa)	Blocks:	
					EA rosette	Adhesion
α_M	OKM1	IgG2a	++++	42, 130, 165	Yes	No
	Anti-Mo1	IgM	++++	165	No	No
	MAb 17	IgM	+++	ND	ND	Yes
	MAb 44	IgG2a	+++	ND	ND	Yes
	Mn41	IgG1	+++	ND	ND	ND
	OKM10	IgG2a	++	ND	ND	ND
	M1/70	IgG2a	++	ND	ND	ND
	Mac-1	IgG2b	ND	ND	Yes	ND
α_X	BU-15	IgG1	++++	165 ± 15	ND	ND
β2	TS1/18	IgG	0	ND	ND	ND
	MHM23		0	ND	ND	ND
CR2	HB5	IgG2a	0	ND	Yes	ND
	Anti-gp140	Polyclonal	0	ND	Yes	ND
	Anti-B2	IgM	0	ND	No	ND

[a] Data were compiled from references 5, 30, 33, 39, 48, and 53. ND, not determined.

provided by Heidenreich and Dierich (45), who determined that iC3b- and C3d-coated sheep erythrocytes formed rosettes with germ tubes and pseudohyphae of *C. albicans* and *C. stellatoidea*. The interaction was both species specific and ligand specific in that *C. tropicalis*, *C. parapsilosis*, and *C. krusei* did not bind iC3b- or C3d-coated erythrocytes; moreover, *C. albicans* and *C. stellatoidea*, which did bind these ligands, failed to form rosettes with C3b-coated erythrocytes. Mannan, D-galactose, L-mannose, and *N*-acetyl-D-glucosamine in concentrations of >65 mM failed to inhibit the adherence of iC3b-coated erythrocytes, whereas D-mannose and D-glucose reduced adherence by approximately 30%.

Antigenic similarity between undefined *C. albicans* components and human complement receptors was demonstrated by other investigators with MAbs specific for epitopes on human integrins (27). Importantly, anti-Mo1, a MAb recognizing an epitope on α_M (CR3), bound to pseudohyphae of *C. albicans*, while anti-B2, a MAb recognizing human CR2, failed to bind to yeast cells. Of some concern, however, is the fact that at least three MAbs recognizing α_M and one recognizing the β2-chain failed to block rosetting of iC3b-coated erythrocytes. With C3d-coated erythrocytes in the assay, the CR2 MAbs HB-5 and anti-gp140 inhibited binding, but at concentrations approaching 500 μg/ml (27). Nevertheless, these studies have been interpreted as demonstrating that *C. albicans* and *C. stellatoidea* bear surface antigens that are immunologically related to α-chain epitopes of CR3 and, to a lesser extent, CR2.

Other investigators (37), using quantitative flow cytometry to confirm the relationship of candidal surface proteins to α_M and α_X (CR3 and CR4), performed binding curves with purified iC3b to establish an association constant (K_a) of 2.45 × 10^6 L/mol, a determination quite similar to that already published for α_M on the human neutrophil (38). Moreover, these investigators were the first to show that iC3b receptors are present on both yeast and pseudohyphal forms. Growth of several *C. albicans* clinical isolates under conditions that promoted hyphal transformation led to a two- to threefold increase in binding of anti-Mo1, OKM1, or M1/70, all anti-α_M MAbs. However, MAbs recognizing CR1 and CR2 did not bind to any isolate of *C. albicans*, regardless of morphology. Growth of *C. albicans* in the presence of 50 mM D-glucose resulted in a four- to sixfold increase in fluorescence and a significant increase (from 16 to 44%) in ^{3}H-iC3b binding compared with growth in 5 mM D-glucose. Of particular note, iC3b binding proceeded in the absence of divalent cations, a critical difference between iC3b binding by neutrophil integrins and that observed with *C. albicans*. Concomitant assay of iC3b binding and phagocytosis revealed an inverse correlation in that increased expression of noncovalent iC3b binding sites on yeast cells significantly inhibited phagocytosis (37). In later studies of phagocytosis, induction of the integrin analog by growth in D-glucose significantly reduced phagocytosis of *C. albicans* blastospores by normal human neutrophils compared with blastospores grown in equimolar L-glutamate (49). These data provided quantitative confirmation of antigenic similarities between *C. albicans* proteins and human α_M or α_X (CR3 or CR4) and suggested a role for these surface molecules in candidal virulence, i.e., the inhibition of phagocytosis.

Structural and functional studies in which ^{125}I-labeled surface components of *C. albicans* were immunoprecipitated with the MAb OKM1 led to the detection of a single band of 130 kDa with minor bands at 50 and 100 kDa following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (29). These studies provided additional confirmation of the cation-independent nature of iC3b binding by *Candida* species. Importantly, a β-chain of 95 kDa was not visualized. Other investigators used immunoblotting with OKM1 to detect a band at 165 ± 15 kDa in cytosolic extracts of *C. albicans* (49). Recently, proteins of 70, 66, 55, and 42 kDa have been isolated from disrupted pseudohyphal forms of *C. albicans* by affinity chromatography with C3(H_2O), a biochemically modified C3 molecule that is conformationally similar to C3b and iC3b (1). The investigators did not identify the 130-kDa protein visualized in previous immunoprecipitation experiments (29). Chromatography of disrupted pseudohyphae on bovine serum albumin-Sepharose revealed proteins of 66 and 55 kDa, among others. Three proteins (66, 55, and 42 kDa) cross-reacted with OKM1 on Western blotting (immunoblotting), but isotype controls for nonspe-

cific binding of MAbs were not included. In ligand-binding assays, the 42-kDa protein bound C3(H_2O), C3b, and iC3b, the 55-kDa protein bound C3(H_2O) but reacted only weakly with C3b and iC3b, and the 66-kDa protein failed to bind any C3 ligand. The investigators hypothesized that the 66- and 55-kDa proteins represented heavily glycosylated forms of the 42-kDa protein (1), but this explanation does not appear to account for the reported differences in ligand binding or for isolation of the 66- and 55-kDa proteins, but not the 42-kDa protein, from bovine serum albumin-Sepharose. Nevertheless, the early identification of a single protein band of 130 to 165 kDa suggests a structural relationship with human α_M or α_X (M_r, 150,000 to 165,000) but not with the β-chain (Fig. 3). In contrast, the 42-kDa protein does not accord with the structure of human α_M or α_X, nor is it clear whether the 66-, 55-, and/or 42-kDa proteins derive from the 130-kDa protein reported previously by these investigators (29). Sequencing of the amino terminus or internal peptides and isolation of the gene(s) will be required to show the derivation of the 42-kDa protein and its relationship to candidal integrin analogs.

Two other well-recognized differences between the human integrins and the candidal proteins should be emphasized. First, there is no evidence of a β2 protein (95 kDa) in *C. albicans* blastospores. The possibility remains that a β-chain, if present, may be expressed in germ tubes or pseudohyphae. In only one study have the data suggested the possibility of a β1 integrin in *C. albicans* (68). In that study, a polyclonal antiserum to the carboxy-terminal domain of the chicken integrin β1 subunit reacted with a crude extract of *C. albicans* hyphae. Second, the binding of iC3b-coated erythrocytes or purified iC3b to *C. albicans* blastospores and hyphae is not cation dependent.

The possible role of integrin analogs in candidal virulence has derived from three separate lines of evidence: (i) the direct correlation between candidal virulence and expression of the integrin analog, since there is little or no expression of integrins on the least pathogenic *Candida* spp.; (ii) the role of the integrin analog in candidal attachment to epithelial and endothelial surfaces; and (iii) the correlation of integrin expression and inhibition of phagocytosis. In most clinical studies, *C. albicans* has been isolated most frequently (60 to 80% of the time), while isolations of *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* follow in descending order (55, 73). *C. albicans* is typically the most adherent yeast species in vitro as well, whether for exfoliated buccal and vaginal epithelial cells, epidermal corneocytes, intestinal epithelial cells, or cultured human epithelium, while *C. tropicalis* ranks second (4, 5, 57, 59, 85). *S. cerevisiae* and other *Candida* species including *C. parapsilosis*, *C. guilliermondii*, *C. krusei*, or *C. pseudotropicalis* typically show little adhesion. Expression of the integrin analog by *Candida* species conforms to a similar hierarchy, as determined by iC3b rosetting (27, 45) or by flow cytometry with α_M or α_X MAbs (4). On flow cytometry, clinical and laboratory isolates of *C. albicans* are the most avid binders of integrin MAbs, whereas *C. tropicalis* isolates are a distant second, and *C. parapsilosis*, *C. glabrata*, *C. krusei*, and *S. cerevisiae* isolates are typically below the limit of detection. Binding of integrin MAbs occurs with *C. albicans* cells incubated in a wide variety of media, including Lee's medium, yeast extract-peptone-dextrose (a synthetic medium), and a basal medium (6, 99, 104). Evidence for the expression of the integrin analog in vivo is derived from immunofluorescence studies in which multiple forms of *C. albicans* were recovered from the peritoneal cavity of mice and stained with OKM1. As can be seen from Fig. 4, there is obvious immunofluorescence on yeast cells, germ tubes, and pseudohyphae (52).

Inhibition studies with integrin MAbs, ligands, and RGD peptides have provided additional evidence for the role of integrin analogs in epithelial adhesion by candidal species. As summarized in Table 2, despite the binding of a wide variety of integrin MAbs by *C. albicans*, only two inhibited epithelial adhesion: MAb 17 (immunoglobulin M [IgM]) and MAb 44 (IgG2a) (5). The C3 fragment iC3b, which is now considered the ligand for the integrin analog, as it is for α_M and α_X on mammalian cells (88, 111), also inhibited adhesion to cultured epithelial or endothelial cells (5, 33, 39). In support of these data, four peptides derived from the RGD site and flanking sequences in iC3b have also inhibited epithelial adhesion for *C. albicans*, while peptides encompassing the RGD site in fibronectin have been, without exception, ineffective in inhibiting epithelial adhesion, in contrast to their effects on endothelial adhesion (5, 62). Finally, it has been demonstrated recently that HeLa cells synthesize and secrete iC3b (74) and that anti-C3 antibodies inhibit the adhesion of *C. albicans* to HeLa monolayers. These separate lines of investigation provide solid evidence that integrin analogs on the surface of *C. albicans* recognize the RGD site in the iC3b molecule synthesized by epithelial cells. Other yeasts, such as *C. parapsilosis*, *C. glabrata*, *C. krusei*, and *S. cerevisiae*, fail to express the integrin analog and therefore lack the "adhesion advantage" that it confers.

The lack of mutants of *C. albicans* deficient only in integrin expression constitutes a handicap in studies of integrin-mediated adhesion, but this can be overcome by genetic techniques once the genes are isolated. Two mutants that have defects involving adhesive properties have been investigated (34, 82). A spontaneous cerulenin-resistant mutant, strain 4918-10, readily bound C3d-coated erythrocytes, but rosetting with iC3b-coated erythrocytes was reduced by more than 50% (82). Both a hyphal homogenate and a culture filtrate from the wild-type strain inhibited the formation of rosettes with iC3b-coated erythrocytes. A macromolecular complex of proteins ranging from 50 to 71 kDa extracted from the parent strain was detected by Western blotting, using an antiserum from a patient with chronic mucocutaneous candidiasis, but the mutant strain lacked the proteins at 50 and 55 kDa under reducing conditions (82). In a collaborative study with our laboratory, flow cytometry for OKM1 was not diminished, nor was adhesion to HeLa cells inhibited when the 4918-10 mutant was compared with its parent strain and a wild-type clinical isolate (3).

The second mutant (A9V2) was selected on the basis of its reduced agglutination with a polyclonal anti-*Candida* antiserum (34). Proteins of 55 to 60, 80 to 84, 115, and 165 kDa were solubilized from cell extracts of the parent strain (A9) by zymolyase and β-mercaptoethanol, but the mutant strain (A9V2) lacked these proteins and was similarly deficient in adhesion to human buccal epithelial cells. Therefore, proteins of 55 to 60 kDa and 165 kDa may be involved in adhesion.

Thus, the participation of integrin analogs in epithelial adhesion in *C. albicans* has been confirmed by identifying a protein in the range of 130 to 165 kDa; by blocking its activity with specific inhibitors such as the integrin MAbs 17 and 44, the ligands iC3b, and RGD peptides; and by defining the epithelial ligand (the RGD site and flanking residues in iC3b). Additional evidence for integrin analogs as virulence factors derives from their role in the inhibition of phagocytosis (37, 49). In early studies, blockade of the integrin

FIG. 4. Photomicrograph of *C. albicans* removed from the peritoneum of fungemic mice and incubated with MAb OKM1. There is visible immunofluorescence surrounding both blastospores and germ tubes (52).

analog on *C. albicans* blastospores with the IgM MAb, anti-Mo1, significantly improved the phagocytosis of yeast cells by normal human neutrophils (37). In order to avoid the confounding effects of Fc-mediated phagocytosis, IgG antibodies were not employed in these experiments. Later, the same investigators grew *C. albicans* blastospores in the presence of 20 mM D-glucose, which increases expression of the integrin analog, or equimolar L-glutamate, which has a negligible effect on expression, and compared phagocytosis of the treated cells (49). In each case, isolates with increased expression of the integrin analog were less avidly phagocytized. The integrin analog is thought to inhibit phagocytosis by virtue of its competition with neutrophil CR3 for iC3b. However, to obtain definitive proof that integrins exist in *C. albicans* and play a role in candidal adhesion and pathogenesis, we must await the cloning of genes encoding integrin-like proteins in *C. albicans* and the development of specific mutants.

Fibronectin receptor. The possibility that a fibronectin receptor mediates epithelial adhesion of *C. albicans* has also been raised, but no experimental evidence has been presented to suggest a relationship with the β1 integrins that function as fibronectin receptors among mammalian proteins. The binding of fibronectin by *C. albicans* and other species was first reported by Skerl et al. (100), who deposited radiolabeled yeast cells in fibronectin-coated microtiter wells. By counting the radioactivity in washes of the wells, the investigators determined that approximately 30 to 40% of *C. albicans* cells adhered in a calcium-dependent fashion; adhesion of *C. tropicalis* was slightly greater when tested side by side with *C. albicans*, but *C. krusei*, *C. pseudotropicalis*, and *S. cerevisiae* failed to adhere to fibronectin. Adhesion of *C. albicans* was inhibited by pretreatment of yeast cells with proteolytic enzymes or by preincubation of fibronectin with yeast mannan. Preincubation of fibronectin with selected monomeric sugars or amino sugars did not result in decreased adherence. In keeping with the observation that buccal and vaginal epithelial cells stain with rabbit anti-fibronectin antibodies, pretreatment of yeast cells with fibronectin decreased adhesion to buccal or vaginal epithelial cells by 40 to 50%. The authors concluded that surface proteins of *C. albicans* and *C. tropicalis* mediated adhesion of yeast cells to fibronectin on epithelial surfaces (100). However, the specificity of this interaction and any potential distinctions between bound and fluid-phase fibronectin were not further delineated. A considerably larger body of work has begun to delineate the participation of a fibronectin receptor in endothelial adhesion (see below).

Lectin-Like Interactions

Adhesive mannoproteins. The interaction of mannoproteins of *C. albicans* with fucose-containing moieties on epithelial cells has received considerable attention and provides an intriguing explanation for the correlation of candidal carriage and blood group O (12, 102). Hosts with blood group

O express the H antigen, a glycoside containing D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, and an α-1,2-fucose moiety, on their buccal and vaginal epithelial cells. A crude mannoprotein preparation from budding yeasts grown in 500 mM galactose inhibited adhesion of *C. albicans* to epithelial cells; the inhibitory effect was strain specific (72). Moreover, treatment of the crude extract with heat, dithiothreitol, or several proteolytic enzymes other than papain abrogated its inhibitory activity, whereas treatment with sodium periodate, α-mannosidase, or endoglycosidase H was ineffective (22). Thus, the adhesive epitope apparently resided in the protein component.

Recently, coupling of blood group determinants (Lewis^a, Lewis^b, and H) to an affinity column has permitted the characterization of a presumed mannoprotein adhesin from *C. albicans* GDH2346 (105). Although the mass of this protein was not cited, the purified preparation was 200 times more active than a crude extract in inhibiting adhesion to buccal epithelial cells. The interaction of lectin-like mannoproteins on *C. albicans* and fucosylated antigens on buccal or vaginal epithelial cells could explain the well-known predilection of nonsecretors and hosts with blood group O for oral or vaginal candidiasis (102). Nonsecretors, in turn, bear the Lewis^a antigen, with L-fucose attached to *N*-acetyl D-glucosamine. Pretreatment of Lewis^a and Lewis^b cells with a Lewis^a antiserum significantly inhibited adhesion of the *C. albicans* strain to those cells (70). Moreover, it would appear that *C. albicans* GDH2346 expresses a lectin specific for L-fucose, while another strain, GDH2023, recognizes *N*-acetyl-D-glucosamine (22, 23). Lectin-like proteins recognizing D-mannose have also been proposed. Isolation of the genes encoding these proteins and their comparison with lectins from plants or more sophisticated eukaryotic cells will clearly provide new insights into the role of lectin-like proteins in candidal adhesion.

Incompletely Defined Interactions

Secretory aspartyl proteinase. The role of extracellular (secretory) aspartyl proteinase in epithelial adhesion has not been completely delineated, despite the purification of the proteinase, the sequencing of the related genes, and several studies corroborating decreased virulence in proteinase-deficient strains (7, 36, 50, 65, 78, 80, 90–92, 103, 110). Aspartyl proteinase has a mass of 42 to 45 kDa and an isoelectric point of 4.4 and is inhibited by pepstatin (90). Genes encoding secretory aspartyl proteinase have been cloned in *C. albicans* and *C. tropicalis* (36, 50, 103, 110). There appear to be two genes encoding aspartyl proteinase in two laboratory strains studied, and both are expressed (110). Sequence analysis and cross-hybridization studies have confirmed 57 to 73% identity among genes from *C. albicans* and *C. tropicalis*. The salient question is whether the proteinase is actually involved in candidal attachment and/or invasion. Evidence implicating a role for aspartyl proteinase includes the demonstration of the proteinase on both blastospores and invading germ tubes of *C. albicans* and *C. tropicalis*, but not *C. parapsilosis*, by immunoperoxidase staining of oral mucosa during experimental infection (7). Moreover, pepstatin, an inhibitor of aspartyl proteinase, prevented the formation of cavitations surrounding blastospores already adherent to murine epidermal corneocytes but did not block candidal adhesion or invasion per se (86). A clear delineation of the true role of aspartyl proteinase in candidal attachment and invasion will likely result from the isolation of "knockout" mutants deficient only in aspartyl proteinase activity and from the employment of assays that distinguish adhesion from invasion.

Factor 6. A second type of incompletely defined interaction is exemplified by factor 6, an epitope in the outer chain of serotype A candidal mannoprotein which is composed of the terminal side chains of mannose residues, bound via β linkage to inner branched structures. A mutant lacking factor 6 was derived from a serotype A *C. albicans* strain and displayed reduced adhesion to a human squamous cell oral carcinoma cell line (75). More recently, several factor 6-deficient mutants isolated with an agglutinating MAb against antigen 6 have shown reduced adhesion to exfoliated buccal epithelial cells and to a human buccal cell line (76). Adhesion to the human buccal cell line was interrupted by a crude mannan extract and by polyclonal antibodies and MAbs. However, the epithelial ligand for factor 6 has not yet been identified. The presence of factor 6 and its putative role in adhesion may account for the predominance of serotype A isolates (68 to 96%) in epidemiologic surveys of *C. albicans* strains in the United States, Europe, and Canada (2, 26, 40). Although most investigators have found a higher incidence of the A serotype, Brawner and Cutler found that serotypes A and B were isolated with equal frequency from immunologically normal hosts (11).

ENDOTHELIAL ADHESION

Comparison of Epithelial and Endothelial Adhesion

Several aspects of epithelial and endothelial adhesion are summarized in Table 3, and a number of these distinctions warrant additional emphasis. First, in contrast to epithelial adhesion, which typically occurs at the point of initial contact of the blastospore with the host, endothelial adhesion arises as a late consequence of the organism's having invaded the submucosal vessels or having been inoculated via an intravascular catheter. Second, epithelial adhesion is predominantly a calcium-independent process, whereas endothelial adhesins are largely calcium dependent, save for the integrin analog. Third, interference with epithelial adhesion could conceivably interrupt or decrease candidal colonization, thereby reducing the risk of fungemia; in contrast, inhibition of endothelial adhesion would prevent the formation of metastatic abscesses in tissue but not bloodstream invasion. Thus, adhesion in *C. albicans* is not only species specific but also tissue specific.

Protein-Protein Interactions

The endothelial adhesins defined to date have been exclusively proteins, and integrin analogs or other candidal surface proteins that recognize integrin ligands (e.g., RGD-containing proteins) appear to play a pivotal role in these interactions. In addition to the integrin analog and its antigenic relationship to the α-chains of the β2 integrin subset, three other proteins that may be related to mammalian integrins have been identified, i.e., the fibronectin receptor (a possible β1 integrin), the laminin receptor (a possible β1 integrin), and fibrinogen-binding proteins (a possible β2 or β3 integrin).

Integrin analog. The role of the integrin analog in mediating the binding of *C. albicans* to human endothelium has been demonstrated in studies with umbilical vein endothelium (39). In this model, specific adhesion again ranged from 20 to 30% and was significantly augmented by growth of yeast cells in the presence of 20 mM D-glucose versus 20 mM

TABLE 3. Comparison of epithelial and endothelial adhesion of *Candida* spp.

Parameter	Epithelial adhesion	Endothelial adhesion
Stage of adhesion	Point of first contact	Late phase after dissemination
Adhesin:ligand	(A) Integrin analog: iC3b (B) Lectins: glycosides (C) Aspartyl proteinase?: ND (D) Factor 6?: ND	(A) Integrin analog: iC3b (B) Fibronectin receptor: fibronectin (C) Laminin receptor?: ND[a] (D) Fibrinogen-binding proteins?: ND
Cation dependent	No	Yes, except for integrin analog
Expression dependent on carbon source in medium	Yes for all but factor 6	Yes for integrin analog; unknown for others
Therapeutic goal	Prevention of colonization	Limitation of tissue metastases

[a] ND, not determined.

L-glutamate. Growth of *C. albicans* in 20 mM D-glucose also correlated with an increased expression of the integrin analog as quantitated by flow cytometry with two α_M MAbs, anti-Mo1 and OKM1. In addition, two distinct MAbs against α_M, MAb 44, an IgG2a antibody, and MAb 17, an IgM antibody, inhibited the adhesion of *C. albicans* to human umbilical vein endothelium by 44 and 71%, respectively. These results demonstrated clearly that endothelial adhesion of *C. albicans* was epitope specific and not isotype specific. In additional experiments, incubation of blastospores with purified human iC3b significantly decreased *C. albicans* adhesion to endothelial monolayers (39).

Support for a role for the integrin analog in endothelial adhesion was presented in a recent publication in which affinity chromatography on concanavalin A-Sepharose was used to separate mannose-containing fractions of hyphal extracts from nonmannosylated proteins (28). The investigators found that the nonmannosylated extract, which contained proteins of 25,000 to 95,000 M_r, blocked adhesion of *C. albicans* to monolayers of human umbilical vein endothelium and reacted on Western blotting with the MAb OKM1. These proteins also reacted with a polyclonal antiserum, CA-A, which recognizes the C3d receptor in *C. albicans* (15, 34, 35, 53, 109). In contrast, mannosylated proteins did not block endothelial adhesion or react with OKM1. Although the C3d receptor is thought to be involved in adhesion to plastic rather than cells (54), the investigators proposed a possible role in endothelial adhesion as well.

Fibronectin receptor. Initial studies of endothelial adhesion suggested a prominent role for fibronectin as ligand since *C. albicans* and *C. tropicalis* adhered to fibronectin in cell culture wells (97, 98, 100). Indirect immunofluorescence disclosed the presence of fibronectin on aortic valves in a rabbit model of nonbacterial thrombotic endocarditis (100). Because the yeasts and bacteria most frequently involved in endocarditis were also the most adherent in vitro, the authors hypothesized that fibronectin on the valvular surface could mediate microbial adhesion in endocarditis.

Using sections of porcine aorta in cell culture wells, Klotz and coauthors confirmed the observation that *C. albicans* and *C. tropicalis* were more adherent to endothelium than were other *Candida* spp., such as *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, and *C. glabrata* (59). These investigators counted nonadherent yeasts that washed from the wells and assumed that yeasts not appearing in the washes adhered specifically to endothelium. In contrast to many studies in this field, viability of yeast cells appeared not to be a major determinant of this interaction, since formalin-killed cells adhered just as well as live organisms, and heat killing reduced adhesion by only 20%. Performance of the assay in the presence of homologous serum reduced adhesion by 28%, despite the absence of *Candida*-specific antibodies. This finding might indicate that free fibronectin in serum could occupy fibronectin receptors on yeast cells and prevent their attachment to fibronectin in the endothelial matrix. However, the caveat here is that adhesive epitopes on free fibronectin are thought to differ from those in cellular matrix. A second distinction from observations with epithelium is that endothelial penetration was observed independently of the production of germ tubes; killed yeast cells and viridans streptococci, although adherent, failed to penetrate vascular endothelium. With *C. albicans*, endothelial damage was limited to the site of candidal attachment, as assessed by scanning and transmission electron microscopy.

Studies of the preferential adhesion of *C. albicans* and *C. tropicalis* to subendothelial matrix proteins, as opposed to endothelium itself, were made after intriguing initial observations (63). *C. albicans* and *C. tropicalis* adhered more avidly to the contracted monolayer, which exposed subendothelial extracellular matrix, than to the confluent monolayer of bovine aortic endothelial cells (60). Treatment of the monolayer with forskolin, which results in spindle-shaped endothelial cells but does not expose the extracellular matrix components, decreased adhesion by approximately 26%, thereby confirming that candidal adhesion was regulated by the availability of ligand rather than by the shape of the endothelial cell. In this model, however, maximal adhesion of *Candida* species was quite low, ranging from 10 to 20% of added yeast cells, significantly less adhesion than had been observed with purified fibronectin in other assays. Because chemotherapeutic agents or endotoxin can lead to exposure of subendothelial matrix in vitro (83), these data provided an interesting insight into the susceptibility of the immunocompromised host to invasive candidiasis.

The identification of fibronectin receptors on *C. albicans* and their role in mediating yeast adhesion to extracellular matrix provided the logical conclusion to the foregoing studies. In this important paper (61), the binding of fibronectin by one of four clinical isolates of *C. albicans* was shown to be saturable, specific, and reversible, with a linear Scatchard plot and a K_d of 1.1×10^{-8} M. Purified fibronectin; two peptides from the RGD site in the fibronectin cell-binding domain, RGD and GRGDTP; and a control peptide, GRGESP, all inhibited the binding of fluid-phase fibronectin to *C. albicans*, whereas carbohydrates (including D-glucose, α-methyl-D-mannopyrannoside, and D-mannose) did not.

Moreover, *C. albicans* adhered to immobilized components of the extracellular matrix, including type I and IV collagen, fibronectin, and laminin. Interestingly, although fibronectin easily inhibited candidal binding to all four substrates, RGD peptides did not reproducibly inhibit binding to collagen or laminin. The latter result, together with data collected on the efficacy of inhibition by GRGESP, suggests that determinants other than the RGD sequence may be important in fibronectin-mediated adhesion. Although data from these experiments are not in agreement with those that demonstrate no role for a fibronectin receptor in epithelial adhesion (5), there may be an explanation. First, it is possible that integrins in *C. albicans* express an α-chain of the β2 subset (α_M-α_X) and a β-chain of the β1 subset. Additionally, of course, it is possible that the tissue itself, whether epithelium or endothelium, in some way determines the preferential expression of integrin α- and/or β-chains.

Others have proposed that integrin analogs may simultaneously recognize a number of common ligands, including iC3b, fibronectin, laminin, and collagen. This idea seems less tenable for several reasons. First, the large sizes of most of these ligands (fibronectin is a dimer of 440,000 M_r, and iC3b has a mass of approximately 100 kDa) make it unlikely that adhesive domains of several ligands could be simultaneously accommodated on a single protein. Second, Klotz and Smith have shown that RGD peptides do not reproducibly inhibit binding of *C. albicans* to laminin or collagen (61). Last, Bendel and Hostetter have recently demonstrated that integrin analogs in *C. albicans* and *C. tropicalis* effectively distinguish between the RGD site in iC3b and fibronectin in epithelial adhesion (5). In this work, peptides encompassing the RGD site and flanking residues in iC3b inhibited adhesion of *C. albicans* to HeLa cells, whereas peptides from the RGD site in fibronectin did not. The converse was true for *C. tropicalis*. Isolation of a gene(s) encoding integrin-like proteins in both blastospores and hyphal forms will be essential to the resolution of these possibilities.

The effects of RGD peptides in limiting the consequences of endothelial invasion have been analyzed by two groups (62, 96). Klotz et al. determined that a 23-mer commercial peptide, PepTite 2000, inhibited adhesion of *C. albicans* to subendothelial matrix in vivo and to purified fibronectin, laminin, and type I and IV collagen in vitro, while a 6-mer RAD peptide had no effect (62). Although attachment of *C. albicans* cells to rabbit aortic endothelium was not decreased in the presence of the 23-mer RGD peptide, the number of live organisms present in liver, brain, heart, and kidneys of animals pretreated with the RGD peptide was significantly reduced. However, since quantitative blood cultures were not reported, the possibility exists that the RGD peptide entrapped the organisms within the vascular bed by preventing their migration into the tissues. This possibility deserves some consideration because of the observation that there was no difference in fatality rates between peptide-treated and untreated animals.

Similar peptides were used to assess the killing of *C. albicans* in the isolated perfused mouse liver model (96). In these experiments, preincubation of *C. albicans* with fibronectin, PepTite 2000, RGD, or RGDS resulted in an approximately twofold increase in the killing of infused *C. albicans*, while peptides GRGDTP, GRGDSP, GRADSP, and GRGESP did not augment fungal killing. These data suggested that hepatic cells preferentially sequestered *C. albicans* bearing specific RGD peptides, even in the absence of antibody, complement, or other plasma opsonins. The fact that GRGDSP and GRGDTP effectively blocked *C. albicans* adhesion to immobilized extracellular matrix proteins (62) but had no effect on hepatic uptake (96) emphasizes again the tissue specificity of adhesion and the possibility that sequences surrounding the RGD site could regulate binding specificity.

Laminin receptor. Laminin-binding proteins, which in mammals are members of the β1 integrin subset, may also play a role in *C. albicans* adhesion to endothelium. For example, a 69-kDa laminin-binding protein has been described in aortic and microvascular endothelial cells; its expression changes during cell attachment, spreading, and migration (112). It is therefore of considerable interest that Bouchara and colleagues have defined a laminin receptor on *C. albicans* with a K_d of 1.3×10^{-9} M and a distribution of 8,000 binding sites per cell (10). By immunoelectron microscopy, binding of laminin was restricted to the outermost fibrillar layer of the hyphal wall and germ tubes and did not occur on blastospores. Both laminin and fibrinogen significantly decreased the binding of fluid-phase laminin to its receptor, but in contrast to the results of Klotz and Smith (61), fibronectin had no effect. A variety of carbohydrates, including mannose, fucose, and *N*-acetyl-D-glucosamine, were similarly ineffective in blocking laminin binding, but 100 mM D-glucose increased laminin binding significantly. From detergent extracts of *C. albicans* germ tubes, proteins of 68 kDa and a doublet of 60 to 62 kDa bound laminin on Western blotting and were detected with rabbit antiserum to laminin. Notably, these appear to be the same proteins that have been proposed as fibronectin and fibrinogen receptors (8, 9, 61, 106). Again, while it is tempting to speculate that a single candidal protein (i.e., a fibronectin-, laminin-, and fibrinogen-binding protein) may recognize diverse ligands, the data indicate that the ligands are not interchangeable because fibronectin did not inhibit laminin binding. Precise controls for specific binding, as performed in these experiments, are essential for such studies, because it is well known that a variety of plasma proteins can bind to *C. albicans* when similar methods are employed (84). It will also be important to determine whether MAbs for β1 integrins can recognize these laminin receptors; at present, their relationship to mammalian integrins remains to be elucidated.

Fibrinogen-binding proteins. Fibrinogen-binding proteins described in *C. albicans* may be related to mammalian integrins of the β2 or β3 subset, although this possibility has not been formally tested (8, 9, 106). Further, although fibrinogen binding may well be important in endothelial adhesion, the interaction of such proteins with endothelial surfaces has not been evaluated. Binding of fibrinogen, whether determined immunocytochemically or with labeled protein, was 7- to 12-fold greater to germ tubes or mycelia than to blastospores, although formal binding curves with unlabeled ligand were not performed and a Scatchard plot was not determined. Fibrinogen binding was inhibited by a combination of protease digestion and mercaptoethanol treatment but not by each alone. Interestingly, fibrinogen, visualized as complexes with colloidal gold particles, extended from the outer surface through the cell wall and was even detected in germ tube cytosol. This observation suggests that fibrinogen-binding proteins may be synthesized during germ tube formation and then exported transmurally.

Subsequent data have provided evidence for a 58-kDa surface mannoprotein, extractable from yeast cells and pseudohyphae with β-mercaptoethanol, to which plasma fibrinogen binds on Western blotting but which does not react with laminin or fibronectin (17). A polyclonal rabbit

antiserum against this protein has been used to stain kidneys, cutis, or urethral epithelium and demonstrates the presence of the protein in both blastospores and pseudohyphal forms in vivo. However, the antibody did not significantly inhibit binding of fibrinogen to mercaptoethanol extracts in an ELISA. In contrast, removal of O-linked oligosaccharides abolished reactivity with fibrinogen but not with the specific antibody. The investigators concluded that O glycosylation may be responsible for the binding of fibrinogen to the 58-kDa protein (17).

Proteins of 60 to 68 kDa have been putatively identified as fibronectin, fibrinogen, or laminin receptors on hyphae extracted by a variety of techniques. Dissociation constants of 1.1×10^{-8} and 1.3×10^{-9} M have been established for fibronectin and laminin receptors, respectively (10, 61). Compared with the low-affinity binding of iC3b, the interaction of *Candida* proteins with these ligands in extracellular matrix occurs with considerably higher affinity, and this, in combination with their uniform presence on germ tubes and not on blastospores, may simplify purification. However, although the mammalian integrin family numbers among its members receptors for fibrinogen, fibronectin, and laminin, direct evidence for the relationship of these candidal proteins with the mammalian integrins has not been presented. Moreover, although laminin and fibrinogen are constituents of the extracellular matrix, specific interactions between laminin- and fibrinogen-binding proteins in *C. albicans* and endothelial cells have yet to be described.

As with epithelial cells, further support for the importance of integrin-like proteins in endothelial adhesion has come from the study of endothelial ligands. For example, the extracellular matrix of endothelial cells is known to contain a variety of integrin ligands, including fibrinogen, fibronectin, and laminin. Similarly, among several cell types, endothelial cells secrete C3 and display iC3b on their surfaces (108). The fact that the binding of *C. albicans* to human umbilical vein endothelial cells causes release of eicosanoids, including prostaglandin E2 (32), suggests that interactions of *C. albicans* adhesins with protein moieties on human cells initiates complex pathways of intracellular signaling that may ultimately lead to damage or destruction of mammalian tissues.

FUTURE INVESTIGATION

The purpose of this review has been twofold: (i) to evaluate what is known about candidal adhesins for epithelial and endothelial cells, and (ii) to suggest exciting avenues for future investigation. Within the latter category, the observation that protein adhesins on *C. albicans* can be modulated by physiologically relevant environmental molecules such as glucose or iron (39, 49, 77) suggests that such adhesins may be involved in complex pathways of intracellular signaling and metabolism. Similarly, the interaction of candidal adhesins with proteins on the surface of epithelial or endothelial cells or with components of the extracellular matrix suggests as well the importance of mammalian signaling mechanisms in the pathogenesis of tissue damage and destruction mediated by fungal colonization and invasion. The dissection of signaling pathways as they originate at the yeast surface through interactions with mammalian molecules and as they may regulate such potentially diverse but fundamentally important processes as yeast growth, metabolism, and dimorphism is clearly a seminal area for future investigation. Our understanding of epithelial and endothelial adhesion, two processes so intimately related to pathogenesis, has just begun. Diligent investigation will provide new insights into these mechanisms and ultimately improve treatment of candidal infections.

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